Mitosis: **Don't get mad, get even**

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The 'mitotic spindle checkpoint' ensures that, before a cell exits from mitosis, all of its chromosomes are aligned on the spindle to form the metaphase plate. Mad2 is an essential component of this checkpoint system and it binds specifically to unattached kinetochores.

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Systematic sequencing of genomes and cDNAs has revealed that many of the proteins expressed in humans are structurally related to proteins found in budding yeast. In some cases, these related proteins are also functionally conserved — the human gene can substitute for the yeast gene — and such functionally conserved proteins are often involved in basic cell biological processes. More specialized and non-essential processes need not rely on structurally conserved proteins because such processes could have evolved independently in different species. Analysis of the cell cycle in budding yeast and in metazoans has revealed that some — but not all — aspects of cell-cycle progression are similar in these divergent organisms.

The yeast and metazoan cell cycles are fundamentally similar. Transitions between cell-cycle phases are governed by the activation and inactivation of cyclin-dependent kinases, which are structurally and functionally conserved between species [1]. Likewise, replication of DNA is mediated by related proteins in divergent eukaryotic species [2–4]. There are, however, significant differences in other aspects of cell division between yeast and metazoans. Whereas the nuclear envelope of a budding yeast cell remains intact throughout the cell cycle, the nuclear envelopes of most metazoan cells break down during mitosis. Although chromosomes are segregated by attachment to microtubules in all eukaryotes, the DNA moiety of the kinetochores — the site of microtubule attachment — consists of 125 base-pairs of DNA in yeast, whereas human kinetochores contain four orders of magnitude more DNA [5]. So, although the cell cycle is a basic process common to all eukaryotic cells, some features of the cell cycle have changed dramatically during the course of evolution.

One goal of cell-cycle research is to understand how chromosomes are accurately replicated and evenly segregated. As failure in either of these processes could cause birth defects, cancer and other diseases, fulfilment of this goal is also a priority for biomedical research. One major step towards understanding these processes was the demonstration that they are scrutinized by systems that detect errors and arrest the cell cycle at the points at which the errors can be corrected [6]. These scrutiny systems were termed 'checkpoint controls', and some of them have the additional property that they are not essential for normal cell-cycle progression.

The integrity of the genome is monitored at several checkpoints during the cell cycle. The first component of a checkpoint to be identified was the *RAD9* gene of *Saccharomyces cerevisiae* [7]. In wild-type cells, DNA damage leads to a cell-cycle arrest prior to mitosis to allow the damage to be repaired. In contrast, *rad9* mutant cells fail to arrest, divide with damaged DNA, and die as a consequence. However, under favorable growth conditions, *rad9* mutant cells are nearly indistinguishable from wild-type cells. This and other studies indicated that the ability to detect damaged and unreplicated DNA is not strictly essential for cell-cycle progression. It is therefore possible that the various checkpoint systems that maintain the integrity of the genome differ in divergent species. However, cells from patients with the disease ataxia telangiectasia (AT) are defective in delaying cell-cycle progression in response to damaged or unreplicated DNA, and the *ATM* gene, which is responsible for the disease, has been found to be structurally related to the yeast *MEC1* gene, which is involved in parallel checkpoints in yeast [8].

The integrity of the genome is not the only aspect of the cell-division cycle that is scrutinized by checkpoint controls. Yeast and metazoans both have checkpoint controls that detect proper assembly of the mitotic spindle. Until just recently, however, it was not known whether the components of this checkpoint were also conserved between species. Molecular understanding of the spindle checkpoint began with the identification of a class of yeast mutants called 'mitotic-arrest deficient' (*MAD*) [9]. These mutants were identified by an analogous screen to the one that implicated *RAD9* in checkpoint control. When wild-type cells are exposed to microtubule-depolymerizing drugs, assembly of the mitotic spindle is impaired and the cells arrest in mitosis. In *mad* mutants, defects in spindle assembly do not prevent cell-cycle progression, and cell division occurs even though the chromosomes segregate randomly. When the genes responsible for the *mad* phenotype were sequenced, the sequences were not informative, nor were they homologous to previously characterized metazoan genes.

Murray and colleagues were interested in finding *Xenopus* homologs of the yeast *MAD* genes, so that they could use cytoplasmic extracts from frog eggs — which undergo the

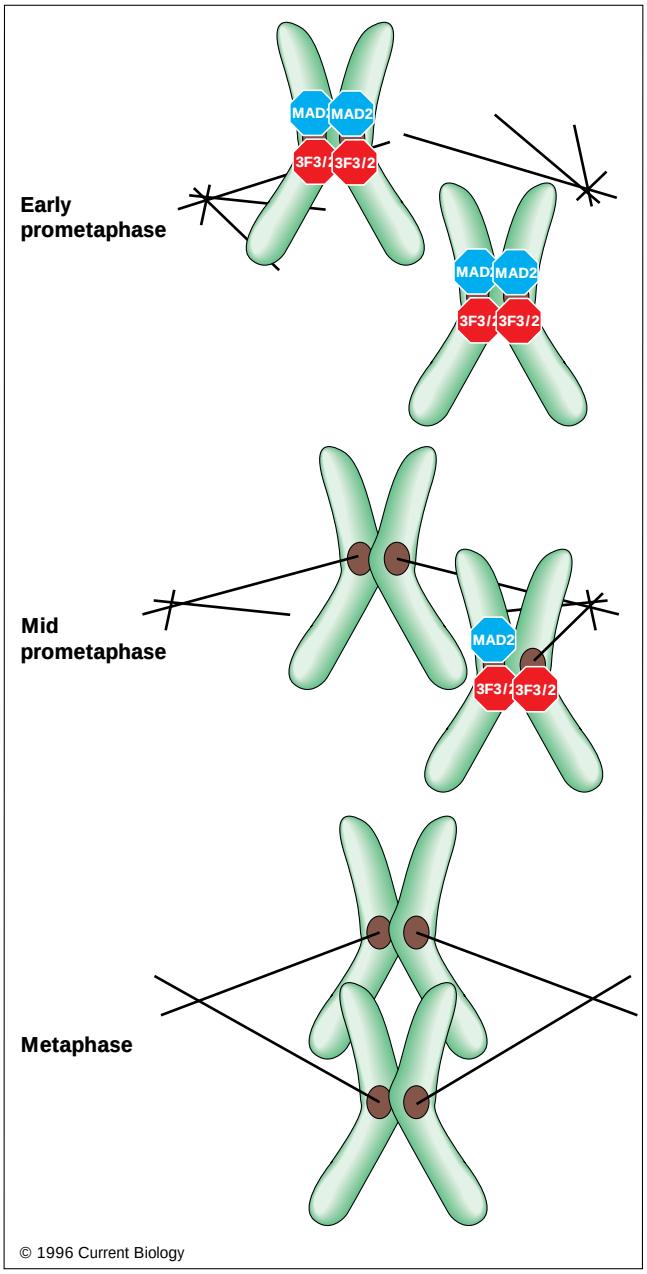
biochemical changes of the cell cycle — to biochemically dissect the mechanism by which Mad proteins detect errors in spindle assembly and arrest the cell cycle. The human and worm genome projects jump-started the search for a *Xenopus MAD* homolog, as each of these sequencing projects uncovered genes with significant homology to the yeast *MAD2*, enabling Chen *et al.* [10] to clone a homologous gene from *Xenopus*, encoding XMad2.

The first priority was to establish whether the XMad2 protein is involved in establishing a cell-cycle arrest in response to spindle defects in *Xenopus* egg extracts. Although early *Xenopus* embryos do not arrest their cell cycles when microtubules are depolymerized, the spindle-assembly checkpoint is active later in development, once the nuclear:cytoplasm ratio has increased. This checkpoint can be reconstituted *in vitro* by supplementing egg extracts with many nuclei [11], and depolymerization of microtubules in such extracts prevents exit from mitosis. When the function of XMad2 was perturbed by the addition of specific antibodies, such extracts lost the ability to arrest in mitosis in response to microtubule depolymerization [10]. Thus, like yeast Mad2, XMad2 is involved in the spindle checkpoint that delays exit from mitosis.

Jumping across experimental systems, from yeast to frog, enabled Chen *et al.* [10] to localize XMad2 in cells in which the resolution of cytological detail is far greater. Detergent extraction of mitotic cells prior to fixation provided a satisfying image: a punctate distribution of XMad2 in the region occupied by the chromosomes. Double-labelling experiments confirmed that these spots corresponded to kinetochores. The kinetochore localization of XMad2 is significant because, in both yeast and higher eukaryotic cells, there is strong evidence that the mitotic spindle checkpoint detects kinetochores which have failed to attach to microtubules [12,13]. In fact, recent experiments have further refined this notion and established that the spindle checkpoint recognizes kinetochores that are not under mechanical tension [14]. Moreover, there is a biochemical difference between kinetochores that are under tension and those that are not. The monoclonal antibody 3F3/2 recognizes a phosphorylated epitope and, in a number of species, 3F3/2 labels the kinetochores of unaligned chromosomes more brightly than those of aligned chromosomes (Fig. 1) [15]. Thus, the presence of bright 3F3/2 staining correlates with operation of the spindle checkpoint. But there are a number of differences between the kinetochores of aligned and unaligned chromosomes, and any of these could, in principle, explain the behaviour of this phospho-epitope. For example, a kinetochore of an aligned chromosome has microtubules bound, it is located in the central spindle, and it is under mechanical tension between the two poles of the spindle.

Nicklas *et al.* [16] performed micromanipulation experiments to distinguish between these possible explanations for the change in the 3F3/2 epitope. They manipulated a chromosome so that it was attached to one spindle pole with both its kinetochores. This monopolar attachment does not generate tension and the kinetochores stain brightly with 3F3/2. However, pulling on these mono-oriented chromosomes in such a way that only one kinetochore was placed under tension caused the 3F3/2 staining to decrease specifically on the kinetochore under

Figure 1



Schematic indicating how microtubule attachment and mechanical tension affect the kinetochore localization of XMad2 and the 3F3/2 phosphoepitope. See text for details.

tension. This demonstrates that down-regulation of the 3F3/2 phosphoepitope is a consequence of mechanical tension. Because XMad2 is essential for the mitotic spindle checkpoint and is localized to the kinetochore, it is possible that XMad2 generates the chemical change that signals improperly aligned chromosomes, or that it transduces this signal to the cell-cycle regulatory machinery.

Detailed analysis of XMad2 localization suggests that the first of these possibilities is more likely. Like the 3F3/2 epitope, XMad2 is present on kinetochores in prometaphase cells and in cells arrested in metaphase by depolymerization of microtubules, but XMad2 is not detectable on the kinetochores of aligned chromosomes [10]. In contrast to the 3F3/2 epitope, however, loss of XMad2 from the kinetochore correlates with attachment of the kinetochore to microtubules, rather than the presence of mechanical tension. These can be distinguished by analysing the staining pattern of chromosomes that are not aligned on the metaphase plate. Non-aligned chromosomes are often attached to one spindle pole *via* microtubules attached to one kinetochore. In these cases, XMad2 is observed only on the unattached kinetochore. This pattern differs from that observed with the 3F3/2 antibody: neither kinetochore in this configuration is under tension and both would stain brightly with 3F3/2. Thus, loss of XMad2 staining appears to precede loss of the 3F3/2 epitope. As XMad2 is required for the mitotic spindle checkpoint, perhaps XMad2 must be present on an unattached kinetochore in order for the 3F3/2 phosphoepitope to be acquired. Interestingly, micromanipulation experiments have shown that reacquisition of 3F3/2 staining at the kinetochore of a previously aligned chromosome requires about 10 minutes. This would suggest that a series of events are required for rephosphorylation of the 3F3/2 epitope, and one such step might be the re-recruitment of XMad2.

XMad2 provides the first evidence that, like the DNA replication checkpoint, the mitotic spindle checkpoint is mediated by homologous proteins in evolutionarily distant organisms. This is a notable finding because it sheds light on the cell cycle of the common progenitor of yeast and metazoans: the conservation of components of the checkpoint in yeast and metazoans indicates that this mechanism was in place before the species diverged. Although loss of the spindle checkpoint in *S. cerevisiae* only modestly increases the rate of chromosome loss under favorable growth conditions, the common progenitor of yeast and metazoans may have had a more error-prone mitotic spindle and may have experienced environmental insults that perturbed spindle integrity. By acquiring an error-detection mechanism, the progenitor species may have increased the efficiency of chromosome transmission to allow for the maintenance of traits that confer a selective advantage.

As with most landmark experiments, the observations of Chen *et. al.* [10] raise more questions than they answer. In particular, what is the biochemical function of XMad2? Is generation of the 3F3/2 phosphoepitope dependent on XMad2? Does the conservation of the kinetochore-based spindle checkpoint indicate that the kinetochores of yeast and humans are more similar than we currently appreciate? Do other eukaryotes — even ones which diverged before the separation of animals and fungi — also contain a Mad2-dependent mitotic spindle checkpoint? In addition, we still want to understand the biochemical events that enable unattached kinetochores to block cell-cycle progression. These questions are likely to prove readily solvable now that the pathway can be dissected by a combination of genetic methods in yeast and biochemical methods in *Xenopus* cell extracts. Progress will be eagerly awaited by all those with an interest in cancer biology, as one important step along the slippery slope of tumor progression may be the loss of the cell cycle's error-checking mechanisms.

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